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How Lysophosphatidylcholine Inhibits Cell–Cell Fusion Mediated by the Envelope

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We have investigated the effect of lysophosphatidylcholine (LPC) on fusion of cells expressing the envelope glycoprotein of HIV-1, gp120/gp41, with cells expressing the receptor for this virus, CD4. Fusion was inhibited by micromolar concentrations of LPC added from an aqueous stock solution, but not by membrane-associated LPC or LPC produced in the membrane by the action of phospholipase A_2. No inhibition was found for LPCs with acyl chain lengths shorter than 12 carbon atoms, and the inhibitory effect of longer molecules increased with their length. gp120-CD4 specific cell–cell binding was inhibited with a chain length dependence corresponding to that of fusion, but with palmitoyl-LPC, fusion was more strongly affected than binding. These data indicate that gp120/gp41-induced fusion is inhibited by LPC because LPC affects viral protein–host cell binding and not because LPC prevented the formation of lipid intermediates required for fusion.

INTRODUCTION

Human immunodeficiency virus (HIV)[2] enters cells by fusion of the viral membrane with the plasma membrane of the host cell, mediated by the HIV glycoprotein complex (Pelchen-Matthews *et al.,* 1995; Larsen *et al.,* 1992; Moore *et al.,* 1993; Freed and Martin, 1995). The complex is composed of two noncovalently linked proteins, gp120 and gp41 (Moore *et al.,* 1993). The latter contains a C-terminal cytoplasmic tail and an ectodomain with a hydrophobic N-terminus, like the fusion proteins of ortho- and paramyxoviruses (Freed *et al.,* 1990; Gallaher, 1987). For example, the influenza virus fusion protein hemagglutinin (HA) is composed of two subunits, HA1 and HA2. Fusion mediated by this protein is triggered by a conformational change in HA resulting in the exposure of a hydrophobic "fusion peptide," located at the N-terminus of the transmembrane subunit HA2 (Hughson, 1995; Bentz, 1993), which is then inserted into the target membrane for fusion (Stegmann *et al.,* 1991; Tsurudome *et al.,* 1992) and thought to play a crucial role in initiating fusion.

Likewise, the N-terminus of gp41 is thought to function as a "fusion peptide" (Larsen *et al.,* 1992; Freed and Martin, 1995), and the conformational changes induced upon binding of gp120 to its receptor on the surface of the host cell (Allan, 1991; Moore *et al.,* 1991; Sattentau *et al.,* 1995) have been suggested to result in the exposure of the fusion peptide (Larsen *et al.,* 1992; Freed and Martin, 1995).

Insertion of fusion peptides in the target membrane is thought to create defects, leading to the formation of transient intermediate lipid structures, such as stalks (Chernomordik and Zimmerberg, 1995; Siegel, 1993a), and finally to the merger of the membranes (Siegel, 1993b). Stalks are highly bent structures composed of fused outer but unfused inner leaflets of the virus and target membrane bilayers. The energy required for stalk formation is determined by the lipid composition of the membranes. Thus, lipids with a large ratio of headgroup area to acyl chain cross-sectional area should inhibit the formation of stalks, if they are present in the outer membrane leaflet (Chernomordik and Zimmerberg, 1995; Chernomordik *et al.,* 1995b). Indeed, lysophosphatidylcholine (LPC), a molecule with such an "inverted cone" shape, was found to inhibit a variety of biological membrane fusion events (Chernomordik *et al.,* 1993, 1995a,c; Yeagle *et al.,* 1994; Vogel *et al.,* 1993), and inhibition of fusion appeared to be caused by inhibition of stalk formation in those cases.

However, we have previously investigated the effect of LPC on influenza virus-induced fusion (Gunther-Ausborn *et al.,* 1995), and found that, in this case, LPC inhibited fusion at a step prior to the formation of lipid intermediates. The inhibitory effect was most likely caused by a direct interaction of the lysolipid with the hydrophobic

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[2] Abbreviations used: FCS, fetal calf serum; HA, hemagglutinin; HIV, human immunodeficiency virus; LPC, lysophosphatidylcholine; LPPC, lyso-1-palmitoyl-*sn*-3-phosphatidylcholine; LMPC, 1-myristoyl-*sn*-3-phosphatidylcholine; LSPC, 1-stearoyl-*sn*-3-phosphatidylcholine; *N*-Rh-PE, *N*-(lissamine rhodamine B sulfonyl) phosphatidylethanolamine; PLA_2, phospholipase A_2; SIV, simian immunodeficiency virus.

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fusion peptide of HA, preventing its insertion into the target membrane.

Here, we have investigated the influence of LPC on cell–cell fusion mediated by the gp120/gp41 protein of HIV-1. Fusion was inhibited only when LPC was added from an aqueous stock solution and remained in the buffer during the incubation. Since LPC either present in the membrane after preincubation of cells with LPC or produced after treatment of cellular membranes with phospholipase A_2 (PLA_2) did not affect fusion, it is concluded that the lysolipid inhibits fusion because it prevents specific cell–cell binding.

MATERIALS AND METHODS

Materials

Cell culture media and fetal calf serum (FCS) were obtained from Gibco (Gibco BRL, Basel, Switzerland). Egg LPC, lyso-1-palmitoyl-*sn*-3-phosphatidylcholine (LPPC), and *N*-(lissamine rhodamine B sulfonyl) phosphatidylethanolamine (*N*-Rh-PE) were purchased from Avanti Polar Lipids (Alabaster, AL). L-methionine sulfoximine, tosyl L-phenylalanin chloromethyl keton-treated trypsin, lyso-1-myristoyl-*sn*-3-phosphatidylcholine (LMPC), lyso-1-stearoyl-*sn*-3-phosphatidylcholine (LSPC), and Triton X-100 were obtained from Sigma. An ammonium sulfate suspension of phospholipase A_2 (PLA_2) from porcine pancreas was purchased from Boehringer-Mannheim.

Cell culture, binding, and syncytium assays

gp120/gp41-expressing CHO K1 cells (CHO-gp160), expressing the full-length *env* of the BH-10 strain of HIV-1 IIIB (Stephens and Cockett, 1989; Bebbington and Hentschel, 1987), and SupT1 cells (a human CD4 expressing the T-lymphoblastic cell line) were grown as described before (Frey *et al.,* 1995). Fibroblasts stably expressing HA (NIH-3T3 HAb-2) (Doxsey *et al.,* 1985) were grown in Dulbecco's modified Eagle's minimal essential medium supplemented with 10% FCS and 2 m*M* L-glutamine. All media contained 100 U/mL penicillin and 100 μg/mL streptomycin.

For fusion induced by gp120/gp41, SupT1 cells (1×10^6) were added to 80–90% confluent CHO-gp160 cells grown on 35-mm-diameter tissue culture dishes or, alternatively, 2.5×10^5 SupT1 cells were added to CHO-gp160 cells grown on coverslips. Samples were incubated in medium for 3 to 5 hr at 37° in the absence or presence of LPC, washed with phosphate-buffered saline (PBS) containing 1 m*M* Ca^{2+} and Mg^{2+}, respectively (PBS^{2+}), to remove unbound SupT1 cells, fixed with ice-cold methanol, and stained with Giemsa as described before (Frey *et al.,* 1995).

For syncytium formation by HA-expressing cells, 80–90% confluent HAb-2 cells grown on 24- or 6-well tissue culture plates were washed and pretreated with tosyl L-phenylalanin chloromethyl keton-treated trypsin (10 μg/mL) for 5 min at 25° to convert the HA to its fusion-active form (Ellens *et al.,* 1990). Proteolysis was stopped by adding serum-containing medium. After 20 min at 37°, the medium was replaced by low pH buffer (135 m*M* NaCl, 15 m*M* sodium citrate, 10 m*M* MES, 5 m*M* Hepes, 1 m*M* $CaCl_2$, pH 4.8) for 3 min at 37° to induce fusion, and the cells were then incubated at neutral pH in serum-free medium in the absence or presence of LPC at 37° for a further 2 to 3 hr. After several washes with PBS^{2+}, fixation with methanol, and staining with Giemsa, a number of cells and nuclei were counted. Fusion was quantitated according to White *et al.* (1981): Fusion index = 1 − (number of cells/number of nuclei).

PLA_2 treatment

Prior to use, PLA_2 was extensively dialyzed against 50 m*M* KCl (pH 8.0) to remove the ammonium sulfate (Romero *et al.,* 1987). Cells were treated with the enzyme (about 140 μg/mL, specific activity 700 U/mg) for 15 to 30 min at 37° in buffer (145 m*M* NaCl, 2.5 m*M* Hepes, 1 m*M* $CaCl_2$) at pH 8.0. The reaction was terminated by adding EDTA to 5 m*M* (final concentration). To determine the extent of phospholipid hydrolysis caused by PLA_2, CHO-gp160 cells were labeled by insertion of a fluorescent phospholipid analogue into the plasma membrane as described by Kok *et al.* (1990). After the reaction, cellular lipids were extracted according to Folch *et al.* (1957) and separated on silica-coated glass plates with chloroform/methanol/25% ammonium hydroxide (70/30/5, v/v/v). To quantify the fluorescence present in the various spots, relevant areas were scraped off from the plate and extracted with several volumes of chloroform/methanol (1/1, v/v), and fluorescence was measured with a Jasco FP777 fluorimeter at an excitation wavelength of 560 nm and an emission wavelength of 590 nm.

Binding experiments

To measure the binding of SupT1 to CHO-gp160 cells, SupT1 cells were metabolically labeled with fluorescent fatty acids as described before (Frey *et al.,* 1995). Briefly, cells were seeded at 1×10^5/mL in medium containing 2 μg of the fatty acid 4,4-difluoro-5,5-dimethyl-4-bora-3a,4a-diaza-3-indacene (BODIPY-FL C11, from Molecular Probes, Eugene OR) per milliliter and grown for 3 days. The cells were washed two times with PBS and incubated in fresh medium containing FCS to remove the remaining nonmetabolized fatty acids. Coverslips with CHO-gp160 cells were placed in 24-well plates, overlaid with 2.5×10^5 labeled SupT1 cells in the presence or absence of LPC and incubated for 3 to 5 hr at 37°. After extensive washing with PBS^{2+} to remove the unbound SupT1 cells, the remaining cells were lysed with 1% Triton X-100 and the fluorescence of BODIPY was measured at an excitation wavelength of 495-nm and an emission

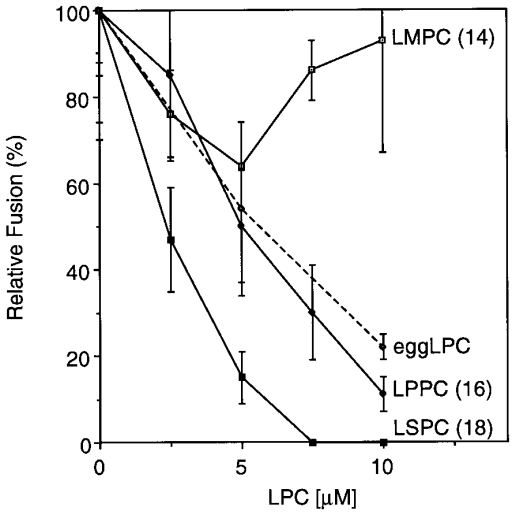

FIG. 1. The inhibitory effect of LPC depends on the hydrocarbon chain length. CHO-gp160 cells, grown on coverslips to 80–90% confluency, were overlaid with 2.5×10^5 SupT1 cells, and different concentrations of egg LPC (dashed line), LMPC, LPPC, or LSPC were added immediately. (Numbers in brackets denote the number of carbon atoms in the acyl chain.) The cells were incubated in serum-free medium in the absence or presence of LPC for 3 hr. After washing with PBS^{2+}, the cells were fixed with methanol and stained with Giemsa. A number of cells and nuclei was counted in several different fields of two coverslips, and for each field a fusion index was calculated as described under Materials and Methods. The fusion indices were averaged; error bars are 1 SD and reflect the difference in fusion indices between the fields. At least 500 nuclei were counted per data point. Fusion indices are expressed as percentage of the control, obtained in the absence of LPC.

wavelength of 512.5 nm. A 495-nm cutoff filter was placed between the sample and the emission monochromator to reduce light scattering. After fluorescence determination, the protein concentration of the samples was measured. Binding was calculated from the fluorescence per milligram cell protein and expressed as a percentage of the control, obtained in the absence of LPC.

RESULTS

Exogenously added LPC inhibits fusion

CHO-gp160 cells, stably expressing the HIV glycoprotein gp120/gp41, are able to fuse with CD4-positive SupT1 cells, resulting in the formation of large syncytia (Frey *et al.,* 1995). Fusion was inhibited when micromolar amounts of egg LPC (mixed acyl chains, from egg yolk) from an aqueous stock solution were added immediately after mixing the CHO-gp160 cells with SupT1 cells (Fig. 1, dashed line).

Because inhibition of fusion in different biological systems was found to depend on the acyl chain length of LPC (Chernomordik *et al.,* 1993, 1995c; Günther-Ausborn *et al.,* 1995), we tested a variety of LPC molecules for their inhibitory activity on gp120/gp41-mediated cell–cell fusion. LSPC (18 carbon atoms) showed the strongest effect on fusion, whereas in the presence of LMPC (14 carbon atoms) there was no effect on fusion up to 10 μM (Fig. 1). LPCs with shorter chains (e.g., hexyl- or decyl-LPC) did not affect fusion at concentrations up to 20 μM (data not shown). Thus, as with influenza virus–liposome fusion (Günther-Ausborn *et al.,* 1995), inhibition of gp120/gp41-induced cell–cell fusion by LPC increased with increasing chain length. For comparison, the effect of LPCs with different chain lengths on influenza HA-induced cell–cell fusion was also determined, as described under Materials and Methods. Syncytium formation between HA-expressing cells was only slightly affected by LMPC, whereas in the presence of LPPC or LSPC fusion was drastically reduced, at about the same concentrations that inhibited gp120/gp41 fusion (data not shown). In conclusion, the chain length dependence of inhibition of gp120/gp41- or HA-induced cell–cell fusion and HA-induced virus–liposome fusion was similar, suggesting a similar inhibitory mechanism. However, for HA-induced cell–cell fusion, as a result of trypsin, low pH, and LPC treatment (see Materials and Methods), many HA-expressing cells had a rounded-up appearance, especially at higher concentrations of longer lysolipids, and the decreased contact between cells probably contributed to the inhibition of fusion.

LPC present in the cell membrane does not inhibit fusion

When LPC is added to membranes from an aqueous stock solution there are three different populations of the lysolipid: monomeric and micellar LPC in the solution and membrane-associated LPC. If the latter causes inhibition of fusion, then preincubation of cells with LPC, leading to its incorporation into the outer leaflet of the membrane (De Kruijff *et al.,* 1977; Bestermann and Domanico, 1992), followed by removal of unincorporated LPC, should also cause an inhibition of fusion. Thus, CHO-gp160 cells were preincubated for 30 min at 37° with 5 or 10 μM LPPC or LSPC, washed once with serum-free medium and overlaid with SupT1 cells. Long chain LPCs like these are not extracted from membranes by washing with buffer (Weltzien, 1979; Chernomordik *et al.,* 1993); thus one can assume that after the wash a certain percentage of lysolipid was still membrane-associated. However, subsequent fusion of the cells with SupT1 cells was not inhibited (Fig. 2), suggesting that the presence of LPC in the medium during incubation of CHO-gp160 and SupT1 cells is a prerequisite for fusion inhibition.

In the above experiment, it is difficult to estimate the concentration of LPC remaining in the membrane. As an alternative, cells were treated with PLA_2, an enzyme which catalyzes the hydrolysis of the acyl chain from the *sn*2 position of phospholipids, to produce lysophospholipids and fatty acids in the membrane. Using fluorescently labeled PE, this approach allowed us to establish

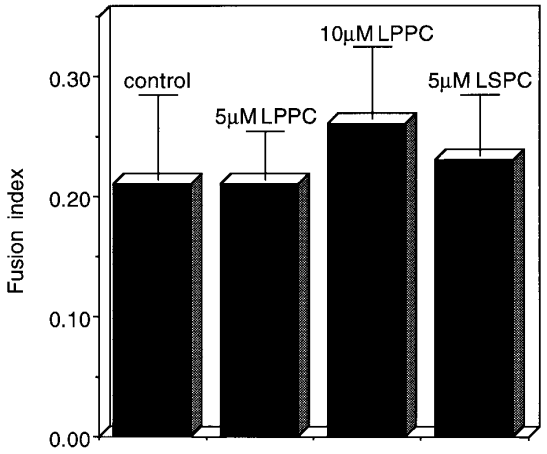

FIG. 2. Fusion after preincubation of cellular membranes with lysolipids. CHO-gp160 cells, grown on coverslips, were preincubated in the absence or presence of LPPC or LSPC for 30 min, 37°. Subsequently, the cells were washed once with serum-free medium and incubated with 2.5×10^5 SupT1 cells for 3 hr. Fusion indices were determined as described in the legend to Fig. 1. Error bars are 1 SD.

that PLA_2 converted phospholipids, present in the outer leaflet of the plasma membrane, into lysolipids. CHO-gp160 cells were incubated with 5 μM (final concentration) fluorescent phospholipid analogue *N*-Rh-PE at 4° for 30 min, leading to its insertion into the plasma membrane of cells (Kok *et al.,* 1990). After extensive washes to remove unincorporated *N*-Rh-PE, cells were incubated for 15 to 30 min with 100 U PLA_2 in the presence of 1 m*M* Ca^{2+} at pH 8.0, 37°. The reaction was stopped by adding 5 m*M* EDTA. The cellular lipids were then extracted and separated by thin-layer chromatography and the fluorescence of *N*-Rh-PE and its hydrolysis product was measured as described under Materials and Methods. In nontreated cells 81 ± 3% of the fluorescence was present in *N*-Rh-PE, and 19 ± 3% was recovered as *N*-Rh-lysoPE, in agreement with previous reports that a fraction of cell-associated *N*-Rh-PE is rapidly degraded to the lyso-form by cellular phospholipase (Kok *et al.,* 1990). However, after incubation with 100 U PLA_2, 93 ± 1% had been converted to *N*-Rh-lysoPE, and only 7 ± 1% of total fluorescence was present in *N*-Rh-PE, indicating that a substantial fraction of the cellular lipids of the outer leaflet had been cleaved by the enzyme.

To test the effect of PLA_2 treatment on fusion, CHO-gp160 cells, grown on 35-mm tissue culture dishes, were incubated with PLA_2 as described above, and after removal of the incubation buffer, cells were overlaid with untreated SupT1 cells in serum-free medium. Alternatively, SupT1 cells were incubated with 100 U PLA_2 as described above, collected by centrifugation, resuspended in serum-free medium, and added to untreated CHO-gp160 cells. As shown in Fig. 3, PLA_2 treatment of either CHO-gp160 or SupT1 cellular membranes did not inhibit fusion. Taken together, these data indicate that LPC present in the outer leaflet of cellular membranes does not inhibit fusion, and therefore, the monomeric or micellar population of exogenously added LPC caused the inhibitory effect.

The latter populations of LPC seemed to inhibit influenza virus–liposome fusion by binding to the fusion peptide of HA, preventing its interaction with the target membrane (Günther-Ausborn *et al.,* 1995). Therefore, if HA-induced cell–cell fusion is inhibited in a similar fashion, LPC produced by PLA_2 should not inhibit fusion. In line with these expectations, HA-induced cell–cell fusion was not inhibited after PLA_2 treatment of cells (Fig. 4). Therefore, the inhibitory effect of LPC on both HA- and gp120/gp41-induced fusion is caused by the monomeric or micellar population and most likely involves lysolipid–fusion protein interactions.

Exogenously added LPC inhibits cell–cell binding

The binding of neighboring cell membranes to each other necessarily precedes their fusion. We recently developed a fluorescence-based assay for the measurement of cell–cell binding (Frey *et al.,* 1995). Fluorescent CD4 expressing cells, grown in suspension, are added to adherent gp120/gp41 expressing cells. After extensive washing, the fluorescence remaining associated with the adherent cells is determined. This assay measures the fluorescence of both bound and fused cells. However, in the presence or absence of an antibody against the V3 loop of gp120, which inhibited cell–cell fusion but not binding, the same amount of fluorescence was found to be associated with the adherent cells (Frey *et al.,* 1995). Thus, the assay can be used to quantitate cell–cell binding. With this assay we measured binding of fluorescent

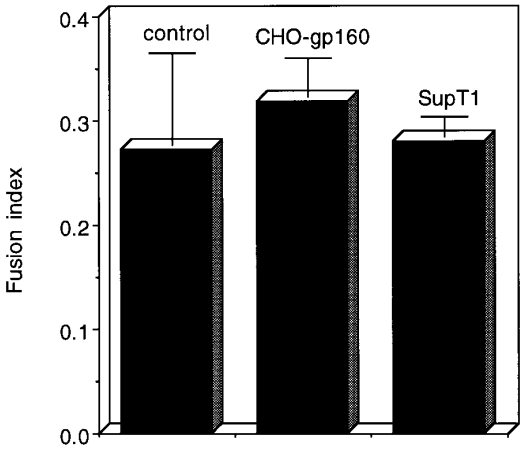

FIG. 3. Lysolipids produced in the membrane do not inhibit fusion. As indicated, either CHO-gp160 cells, grown on 35-mm tissue culture dishes, or 1×10^6 SupT1 cells were incubated with 100 U PLA_2 at pH 8.0 for 30 min at 37°. The reaction was terminated by adding EDTA. Subsequently, PLA_2-treated cells were added to nontreated SupT1 or CHO-gp160 cells, respectively, and fusion was measured in serum-free medium as described in the legend to Fig. 1. In the control, neither cell line was treated with PLA_2. Error bars are 1 SD.

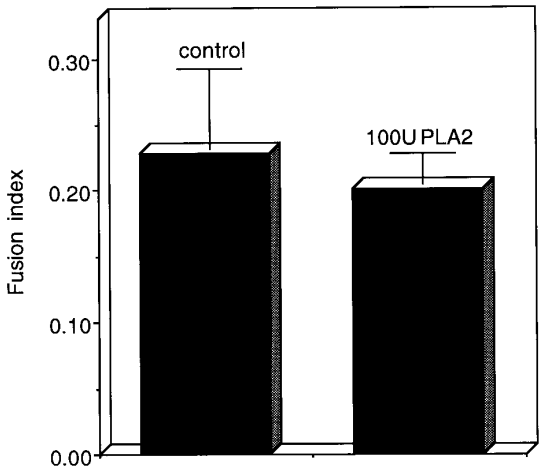

FIG. 4. LPC produced in HA-expressing cellular membranes does not affect fusion. HA-expressing cells, grown on 35-mm tissue culture dishes, were pretreated with 10 μg/mL trypsin for 5 min, 25° followed by an incubation in FCS-containing medium for 20 min, 37° as described under Materials and Methods. Subsequently, cells were washed with serum-free medium and incubated with or without (control) 100 U PLA_2 at pH 8.0 for 30 min at 37°. After low pH treatment (3 min, pH 4.8, 37°), cells were incubated in serum-free medium at neutral pH for 2 hr, 37°. Fusion indices were determined as described in the legend to Fig. 1. Error bars are 1 SD.

SupT1 cells (labeled with BODIPY fatty acids as described under Materials and Methods) to CHO-gp160 cells in the absence or presence of LPC. After removal of unbound SupT1 cells by extensive washing with PBS^{2+} the remaining cells were lysed with 1% Triton X-100 in PBS and the BODIPY fluorescence was measured. As shown in Fig. 5, the gp120-CD4-specific cell–cell binding was reduced in the presence of increasing concentrations of LPCs with longer acyl chains, whereas LMPC did not show an effect at concentrations up to 10 μM. Within the range of concentrations tested, nonspecific binding of SupT1 cells to CHO cells not expressing gp160 was not affected (Fig. 5). At any concentration of LPPC fusion was more inhibited than binding; e.g., binding was inhibited by about 20% in the presence of 5 μM LPPC, whereas fusion was reduced by 50% at that concentration, indicating that there is an effects of LPPC beyond the inhibition of binding. LSPC inhibited both binding and fusion more strongly than LPPC, and there was no difference between the effects on binding and fusion; half-maximal inhibition of binding was found around 2 μM, with 50% fusion being inhibited by 2.3 μM LSPC. Taken together, the above data indicate that LPC inhibits gp120/gp41-induced cell–cell fusion largely because it inhibits CD4-gp120-specific cell–cell binding and other interactions of the viral glycoprotein complex with the host cell membrane.

DISCUSSION

The fusion of any two lipid bilayers requires the formation, at least locally and transiently, of nonbilayer structures (Wilschut and Hoekstra, 1986). Since LPC was found to inhibit fusion events ranging from fusion induced by viral proteins (Chernomordik *et al.,* 1993, 1995c; Yeagle *et al.,* 1994), to cortical granule exocytosis (Vogel *et al.,* 1993; Chernomordik *et al.,* 1993) and fusion between model membranes (Yeagle *et al.,* 1994; Chernomordik *et al.,* 1995a), it was concluded that a step common to many fusion processes, like the formation of these nonbilayer structures, is most likely affected (Chernomordik *et al.,* 1995c). The most plausible current model for membrane fusion features lipid intermediates called "stalks," which are hourglass-shaped structures composed of fused outer but unfused inner leaflets of two interacting membranes (Chernomordik *et al.,* 1985; Siegel, 1993a). The surface of the fused membrane leaflet which forms the stalk has a net negative curvature. The presence of LPC in the outer leaflet of contacting membranes should inhibit the formation of structures with negative curvature and therefore stalk formation (Chernomordik *et al.,* 1995b; Chernomordik and Zimmerberg, 1995). In accordance with this theory, LPC added to the contacting leaflets of pure lipid membranes was found to inhibit fusion (Chernomordik *et al.,* 1995a). Similar results were reported for baculovirus gp64-induced cell–cell fusion: fusion was inhibited by LPC added exogenously or

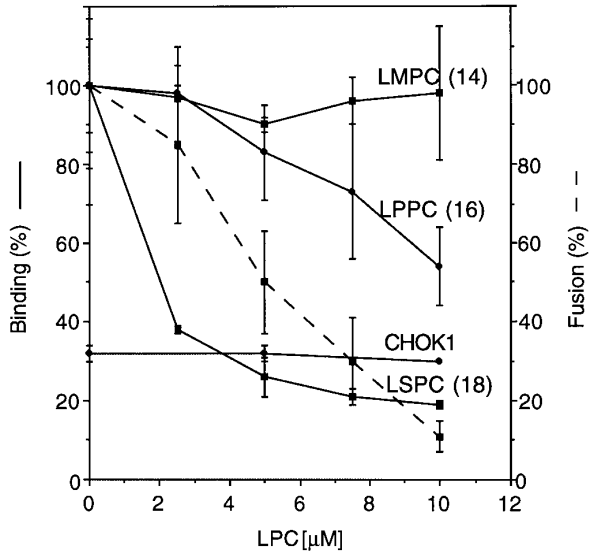

FIG. 5. LPC inhibits cell–cell binding. CHO-gp160 cells, grown on coverslips, were incubated with 2.5×10^5 fluorescent SupT1 cells in the presence of different concentrations of LMPC, LPPC, or LSPC as described in the legend to Fig. 1. After 3 hr, cells were washed several times with PBS^{2+} and lysed with Triton X-100, and the fluorescence was measured as described under Materials and Methods. Binding in the absence of LPC was set to 100% and binding in the presence of LPC was expressed as a percentage of this control. Note that the assay measures the fluorescence of both bound but unfused SupT1 cells and cells that were bound and had already fused with CHO-gp160 cells (Frey *et al.,* 1995). Nonspecific binding of SupT1 cells to CHO cells not expressing gp120/gp41 was also measured. For comparison, the dashed line represents fusion in the presence of LPPC (data from Fig. 1). Error bars are 1 SD.

produced in the membrane by PLA_2 treatment, suggesting that baculovirus fusion may proceed through these intermediate structures. Therefore, inhibition of fusion by LPC appeared to provide a useful indication of the involvement of stalks as lipid intermediates.

However, biological membrane fusion is always induced by fusion proteins (Stegmann *et al.,* 1989), and therefore the inhibitory effect of LPC could also be due to lysolipid–fusion protein interactions. It is clear that LPC inhibited gp120/gp41-mediated cell–cell fusion at the level of specific cell–cell binding. The CD4 receptor, which is present on the SupT1 cells that we used as targets, binds the gp120 subunit with high affinity, and the initial binding event between the two molecules appears to involve a hydrophobic interaction between the CD4/CDR2 loop and a hydrophobic region of gp120 (Moore *et al.,* 1993). Binding of LPC to hydrophobic binding sites on gp120 or CD4 could block the sites, thus affecting receptor–ligand interactions and, consequently, inhibit fusion. Moreover, recently the CD4–gp120 complex has been shown to associate with a second receptor present in the membrane of the target cell (Lapham *et al.,* 1996). Although gp120 can interact with this second receptor in the absence of CD4, CD4 binding greatly increases the affinity for the second receptor (Wu *et al.,* 1996; Trkola *et al.,* 1996). Antibodies capable of neutralizing HIV, recognizing epitopes in the V3 loop of gp120, were shown to block the interaction with this second receptor (Wu *et al.,* 1996; Trkola *et al.,* 1996). Thus, it is also possible that LPC inhibits these interactions. However, at any concentration of LPPC fusion was inhibited more strongly than binding, suggesting that postbinding events also were influenced by the monomeric and micellar population of LPC.

Fusion of influenza virus with liposomes was inhibited by LPC at a step prior to the formation of lipid intermediates (Günther-Ausborn *et al.,* 1995). In this case, LPC inhibited binding of the virus to zwitterionic membranes at low pH, and it inhibited the leakage of small molecules from liposomes, both of which are induced by the interaction of the fusion peptide of HA with the target membrane. However, fusion peptide insertion into membranes containing LPC was still possible (Günther-Ausborn *et al.,* 1995). These data seemed to suggest that the hydrophobic part of LPC binds to the hydrophobic fusion peptide, and if this is covered with LPC it can no longer insert into the target membrane. Shangguan *et al.* (1996) also found that HA-mediated fusion was inhibited by LPC and suggested that LPC–HA interactions could be affected, rather than lipid intermediates.

HIV and simian immunodeficiency virus (SIV) appear to have fusion peptides at the N-terminus of their transmembrane protein (Larsen *et al.,* 1992; Freed and Martin, 1995). Mutations in these peptides affected the fusion capacity of these proteins (Felser *et al.,* 1989; Bosch *et al.,* 1989; Kowalski *et al.,* 1987; Freed *et al.,* 1990, 1992; Steffy *et al.,* 1992; Martin *et al.,* 1996).

Therefore, one could speculate that LPC might inhibit fusion induced by the HIV gp120/gp41 by a mechanism similar to that by which LPC affects influenza fusion. Moreover, LPC could more subtly alter the interaction between fusion peptides and target membranes, for example by affecting the orientation of this peptide in the target membrane (Martin *et al.,* 1993; Colotto *et al.,* 1996). In this respect, it is interesting to note that fusion between liposomes and the leakage of small molecules from these liposomes, induced by synthetic peptides corresponding to the N-terminal segment of HIV or SIV, was inhibited also by (exogenous) LPC (Martin and Ruysschaert, 1995).

Experimental distinction between the two mechanisms of inhibition (LPC–protein interactions and an effect on lipid intermediate formation) is easiest if there are outspoken differences between the effect of LPC in solution and that of membrane-associated LPC, as in this paper. The chain length dependence of inhibition is more difficult to evaluate. LPC inhibits the formation of negatively curved structures because its headgroup diameter is larger than its acyl chain diameter (inverted cone shape). Therefore, if fusion is inhibited because stalk formation is inhibited, one would expect shorter LPCs, with a more pronounced inverted cone shape to more strongly affect fusion; this was indeed found in some cases (Chernomordik *et al.,* 1993, 1995c). However, the difference in shape is most pronounced for molecules with a chain length of less than eight carbon atoms (Kumar, 1991), and these did not inhibit fusion in most cases (Chernomordik *et al.,* 1993; Günther-Ausborn *et al.,* 1995). On the other hand, HA- and gp120/gp41-induced fusion were inhibited by the monomeric or micellar populations of LPC, but inhibition increased with increasing chain length. In the presence of membranes, longer chain LPCs partition more easily into the membranes, and therefore the concentration of LPC in the solution decreases with increasing chain length (Brown *et al.,* 1993). Thus, one has to assume that hydrophobic sites in proteins preferentially bind to longer, more hydrophobic LPCs. Similar observations were made for the binding of LPC to bovine serum albumin via hydrophobic interactions (Brown *et al.,* 1993).

The reported concentrations of LPC required for inhibition of cell–cell fusion induced by baculovirus gp64 were about 10-fold higher than those needed for inhibition of HIV or influenza fusion (Chernomordik *et al.,* 1995c; see also published erratum). Differences in experimental setup and type of cells (insect vs mammalian cells) could be responsible for the difference. However, it is also possible that these differences are related to differences in hydrophobicity of the sites in gp120/gp41 and the HA fusion peptide on the one hand and hydrophobic sites in gp64 on the other.

In conclusion, LPC did not inhibit fusion mediated by the HIV gp120/gp41 because it prevented the formation of lipid intermediates, but seemed to act at an earlier stage, as we have found previously for the influenza virus HA. But since LPC inhibition of fusion acts at an early stage of the fusion process, we cannot exclude that lipid intermediates would be inhibited, if they were formed. The data presented in this paper show that inhibition was caused by nonmembrane-bound LPC which affected the interactions of the viral fusion protein with the target membrane. In addition to a direct effect on the gp120–host cell interaction, the postbinding effects, observed with LPPC, could be interpreted to suggest a direct interaction of the lysolipid with the putative fusion peptide of gp41. Such interactions between LPC and hydrophobic parts of fusion proteins could also play a role in other biological fusion events and have to be distinguished from the effect of LPC on the formation of fusion intermediates.

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